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the deduced amino acid sequence of the borage  $\Delta^6$  desaturase (SEQ ID NO:6) and that of C3D6.1 (SEQ ID NO:7) (Fig. 2B). The extra sequence predicted for W08D2.4 is likely derived from incorrect prediction of intron-exon borders.

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(9) At page 22, delete the paragraph from lines 16-18 and substitute the following paragraph:

Note the presence of the H-P-G-G (SEQ ID NO:8) cytochrome  $b_5$  motif in the N-terminus (encoded by bases 96-108) and the H Q substitution in the third histidine box (encoded by bases 1157-1172).

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(10) Please enter pages 1-10 of the sequence listing after page 31 of the application.

### Remarks

#### The Amendments

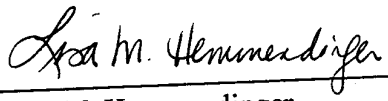
The specification is amended to insert sequence identifiers and to correct minor clerical and grammatical errors. No new matter is added.

#### The Sequence Listing

A paper and a computer readable form of a sequence listing accompany this paper. I believe the contents of the computer readable and paper forms are identical. The sequence listing contains only those sequences disclosed in the application as filed and adds no new matter.

Respectfully submitted,

Date: 2-19-02

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Appendix 1. Version of the amended paragraphs, with markings to show changes made

3  
(1) Page 2, lines 17-18:

According to the present invention, there is provided a polypeptide having desaturase activity, which comprises the amino acid sequence shown in Figure 1 (SEQ ID NO:2).

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(2) Page 2, lines 20-23:

The amino acid sequence shown in Figure 1 (SEQ ID NO:2) is that of a  $\Delta^6$  desaturase that is present in the nematode worm *Caenorhabditis elegans*. This is highly significant since prior to the present invention no successful sequencing or purification of an animal  $\Delta^6$  desaturase had been reported. As *C. elegans* does not accumulate GLA isolation of a  $\Delta^6$  desaturase from it was an unexpected target in which to isolate a desaturase gene [for isolating desaturases gene in].

(3) Page 4, line 25 to page 5, line 2:

The amino acid sequence shown in Figure 1 (SEQ ID NO:2) is also of significance because it has a very low level of sequence identity with the borage  $\Delta^6$  desaturase (the only other eukaryotic  $\Delta^6$  desaturase to have been sequenced prior to the present invention). Indeed, this level of sequence identity is below 32%. At such a low level of identity it might be expected that the two polypeptides would have completely different functions. Unexpectedly, both have  $\Delta^6$  desaturase activity.

(4) Page 3, lines 4-9:

The present invention is, however, not limited to a  $\Delta^6$  desaturase having the sequence shown in Figure 1 (SEQ ID NO:2). It includes other desaturases having at least 32% sequence identity therewith. Preferred polypeptides of the present

invention have at least 40% or more, preferably at least 50% amino acid sequence identity therewith. More preferably the degree of sequence identity is at least 75%. Sequence identities of at least 90%, at least 95% or at least 99% are most preferred.

(5) Page 3, lines 23-29:

In summary, a polypeptide of the present invention has desaturase activity and:]

- a) comprises the amino acid sequence shown in Figure 1 (SEQ ID NO:2);
- b) has one or more amino acid deletions, insertions or substitutions relative to a polypeptide as defined in a) above, but has at least 32% amino acid sequence identity therewith; or
- c) is a fragment of a polypeptide as defined in a) or b) above, which is at least 100 amino acids long.

(5) Page 4, lines 7-15:

Desirably a polypeptide of the present invention will have a cytochrome domain. A cytochrome domain can be defined as an electron-transporting domain that contains a heme prosthetic group. Preferably a cytochrome b domain is present. More preferably a cytochrome b<sub>5</sub> domain is present (desirably this includes a H-P-G-G-X<sub>15</sub>-F-X<sub>3-6</sub>-H (SEQ ID NO:3), where X is any amino acid, motif). A cytochrome b<sub>5</sub> domain is present in both the borage  $\Delta^6$  desaturase and in the *C. elegans*  $\Delta^6$  desaturase amino acid sequence shown in Figure 2B (SEQ ID NO:7). The cytochrome b<sub>5</sub> domain is preferably an N-terminal domain – i.e. it is closer to the N-terminal end of the desaturase than to the C-terminal end. This contrasts with other desaturases. For example, yeast  $\Delta^9$  desaturase[,] has a c-terminal cytochrome b<sub>5</sub> domain and plant  $\Delta^{12}$  and  $\Delta^{15}$  desaturases which do not have any b<sub>5</sub> domain.

(6) Page 8, lines 5-9:

As discussed *supra*, it is often advantageous to reduce the length of a polypeptide. Feature c) of the present invention therefore covers fragments of the polypeptides a) or b) above which are at least 100 amino acids long, but which do not need to be as long as the full length polypeptide shown in Figure 1 (SEQ ID NO:2). Desirably these fragments are at least 200, at least 300 or at least 400 amino acids long.

(7) Page 21, line 21 to page 22, line 4:

A cDNA library and EST yk436b12 were generously provided by Prof Y. Kohara (National Institute of Genetics, Mishima, Japan) and a number of positive plaques were identified by screening with the EST insert. These were further purified to homogeneity, excised, and the largest inserts (of ~1450 bp) from the resulting rescued phagemids were sequenced. This confirmed that the cDNAs isolated by us were indeed homologous to W08D2.4, with the 5' and 3' ends of the cDNA being equivalent to bases 9 and 3079 of the sequence of cosmid W08D2. Since the ATG initiating codon predicted by the Genefinder programme to be the start of gene product W08D2.4 was indeed the first methionine in the cDNA clone, we reasoned that we had isolated a *bona fide* full length cDNA. The DNA sequence and deduced amino acid sequence of one representative cDNA clone (termed pCeD6.1; 1463 bp in length) is shown in Fig 1 (SEQ ID NO:1); the deduced amino acid sequence (SEQ ID NO:2) is identical to that predicted for W08D2.4 over the majority of the protein.

(8) Page 22, lines 6-14:

However, DNA sequences encoding residues 38-67 (Y-S-I . . . L-Y-F) predicted for W08D2.4 are not present in the cDNA clone. This means that the deduced amino acid sequence of CeD6.1 is in fact 443 amino acids long (SEQ ID NO:4).

as opposed to that predicted for W08D2.4, which is 473 residues in length (SEQ ID NO:5). The only other difference between the two amino acid sequences is an M→V substitution at residue 401, resulting from an A→G base change (base 1211). The two sequences are compared in Fig.2A (SEQ ID NOS:4 and 5), as is the deduced amino acid sequence of the borage  $\Delta^6$  desaturase (SEQ ID NO:6) and that of C3D6.1 (SEQ ID NO:7) (Fig. 2B). The extra sequence predicted for W08D2.4 is likely [to] derived from incorrect prediction of intron-exon borders.

(9) Page 22, lines 16-18:

Note the presence of the H-P-G-G (SEQ ID NO:8) cytochrome  $b_5$  motif in the N-terminus (encoded by bases 96-108) and the H Q substitution in the third histidine box (encoded by bases 1157-1172).